Recombinant Human CD39L3/ENTPD3
Catalog Number: 4400-EN

DESCRIPTION

Source
Mouse myeloma cell line, NS0-derived
Gin44-Pro485, with a C-terminal 6-His tag
Accession # O75355

N-terminal Sequence Analysis
No results obtained: Gin44 predicted

Predicted Molecular Mass
50 kDa

SPECIFICATIONS

SDS-PAGE
65-80 kDa, reducing conditions

Activity
Measured by its ability to hydrolyze the 5-phosphate groups from the substrate adenosine-5'-triphosphate (ATP). The orthophosphate product is measured by a Malachite Green Phosphate Detection Kit (Catalog # DY996).

Endotoxin Level
<1.0 EU per 1 µg of the protein by the LAL method.

Purity
>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.

Formulation
Supplied as a 0.2 µm filtered solution in Tris, CaCl₂, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials
- Assay Buffer: 25 mM Tris, 5 mM CaCl₂, pH 7.5
- Recombinant Human CD39L3/ENTPD3 (rhCD39L3) (Catalog # 4400-EN)
- Substrate: ATP (Sigma, Catalog # A-7699), 10 mM in deionized water
- Malachite Green Phosphate Detection Kit (Catalog # DY996)
- 96-well Clear Plate (Costar, Catalog # 92952)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay
1. Dilute rhCD39L3 to 0.02 µg/mL in Assay Buffer.
2. Prepare a standard curve from 1 M Phosphate Standard by adding 10 µL of the 1 M Phosphate Standard to 990 µL of Assay Buffer for a 10 mM stock. Continue by adding 10 µL of the 10 mM Phosphate stock to 990 µL of Assay Buffer for a 100 µM stock. (This is the first dilution to use as a standard).
3. Perform six additional one-half serial dilutions of the 100 µM Phosphate stock in Assay Buffer. The standard curve has a range of 0.039 to 2.5 nmol per well.
4. Load 25 µL of 0.02 µg/mL rhCD39L3 and the standard curve into a plate. Include a Substrate Blank containing Assay Buffer.
5. Dilute the Substrate to 0.02 mg/mL in Assay Buffer.
6. Add 25 µL of the 100 µM Substrate to all wells and mix well.
7. Cover the plate with parafilm or a plate sealer and incubate at 37 °C for 30 minutes.
8. Add 10 µL of the Malachite Green Reagent A to all wells. Mix and incubate for 10 minutes at room temperature.
9. Add 10 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
10. Read plate at 620 nm (absorbance) in endpoint mode.
11. Calculate specific activity:

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\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released} \times (1000 \text{ pmol/nmol})}{\text{Incubation time (min)} \times \text{amount of enzyme (µg)}}
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*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Substrate Blank.

Final Assay Conditions

Per Well:
- rhCD39L3: 0.0005 µg
- Substrate: 35.7 µM

PREPARATION AND STORAGE

Shipping
The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

Ectonucleoside triphosphate diphosphohydrolase-3 (NTPDase-3), encoded by the ENTPD3 gene and also known as CD39L3, is an integral membrane protein with an extracellular active site (1). Recombinant human NTPDase-3 was expressed as a protein lacking its N- and C-terminal transmembrane domains, resulting in the secretion of the soluble ectodomain. NTPDase-3 hydrolyzes the β- and γ-phosphate residues of nucleotides, preferring ATP, ADP, UTP, and UDP as substrates (1).

Through its hydrolysis of extracellular nucleotides, NTPDase-3 plays a role in the regulation of purinergic signaling (2). The enzyme is expressed at its highest levels in brain, pancreas, spleen and prostate tissues (3).

References:

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